SPERMATOPHORE PLACEMENT IN THE COPEPOD
EUCHAETA NORVEGICA BOECK 1872
FROM DEEPWATER DUMPSITE 106

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Abstract.—Numbers and placement of spermatophores on females of *Euchaeta norvegica* from Deepwater Dumpsite 106 in the continental slope waters off Delaware are discussed and compared to those published from Loch Etive, Scotland. Numbers of single, double, and triple placements are comparable but numbers of spermatophores placed away from the ventral face of the genital prominence are higher in specimens from Deepwater Dumpsite 106. Changes in the spermatophore before and after attachment by the male are described. These include the addition of a long stalk, an attachment plate, and in some cases, a fertilization tube. An hypothesis is advanced to explain the imprecision in spermatophore placement.

Introduction

The separate phenomena of multiple spermatophore attachments and misplaced spermatophores, i.e., spermatophores attached away from the immediate area of the genital opening of the mature female, have received some attention in the literature on copepod reproductive behavior. Marshall & Orr (1955) briefly summarized reports for *Calanus finmarchicus*, noting that females occasionally carry more than one spermatophore and that as many as 15 have been reported by Gibbons (1936). Fleminger (1967) reported that females of *Labidocera diandra* had spermatophores attached in 2 positions; one spermatophore (in type 2 position) he considered nonfunctional due to a lack of a connection to the genital pore. Multiple spermatophores were also observed in *L. diandra* with the spermatophores in a variety of combinations in the 2 positions. Multiple spermatophores were reported for laboratory-reared *Eurytemora affinis* (Katona, 1975). Lucks (1937), cited by Katona (1975), noted that multiple spermatophores in *E. affinis* from the Baltic coast could be attached one upon the other or scattered over the ventral surface of the genital segment. Hammer (in press) reports multiple spermatophores on *Acartia tonsa*; these spermatophores are not misplaced but instead set one upon the other. Hopkins & Machin (1977) have reported multiple spermatophores with a variety of misplacements in *Euchaeta norvegica* from Loch Etive, Scotland.

Multiple spermatophores have most often been considered the result of an excess proportion of males to females (Fleminger, 1967; Katona, 1975). Misplaced spermatophores present a more complex question es-

pecially concerning their potential use by the females. Hopkins & Machin (1977) suggest that at least some misplaced spermatophores of *Euchaeta norvegica* are fully viable through a connection to the female genital opening, the fertilization tube. This paper presents data on spermatophore placement, including multiple and misplaced spermatophores, in *Euchaeta norvegica* from the continental slope waters off Delaware.

Materials and Methods

From 23 July to 2 August 1975, samples were collected from the pelagic environment of Deepwater Dumpsite 106 (DWDS), 72°00′–72°30′W and 38°40′–39°00′N, in waters of the continental slope off Delaware. A ten foot Issacs-Kidd Midwater Trawl with a meter plankton net (mesh width 1 mm) on the posterior part was equipped with a discrete-depth cod-end sampler. Further details of the sampling scheme and station positions can be found in Kruger et al. (1977). Although DWDS is usually occupied by slope water, the area is hydrographically quite dynamic (Warsh, 1975; Bisagni, 1976; Morgan & Bishop, 1977). During the sampling period, the hydrography of the area was complicated by the influence of a warm core eddy whose center was situated east of DWDS. In the following analysis no attempt has been made to determine if the observations of spermatophore placement in *E. norvegica* might be correlated with the horizontal and vertical distributions of the animals or affected by the complex hydrography of the area.

Copepods were sorted from 51 stations, each station having from 1–4 discrete depth samples. An analysis of the vertical and horizontal distributions of the copepod fauna is in progress. *Euchaeta norvegica* Boeck, 1872 was the most common and abundant copepod in the collection, occurring in 57 samples from 27 stations. Mature females and males of *E. norvegica* in these samples had begun spermatophore transfer. In addition to reporting the results of spermatophore placement in *E. norvegica* from DWDS 106, I compare this population with those from Loch Etive. It should be noted that Hopkins & Machin (1977) collected 1 sample every 10 days during 11 months of the year. Most of the values I have cited from their paper represent average values. The data in my paper are averages of all samples collected during the 11 day period. In terms of elapsed time, then, the values are comparable to a single sample from Loch Etive.

Figure 1 shows the left and right lateral views of the genital segment of a mature female. In the following description of this segment, I have used the terminology of Park (1975). The dominant feature is the large genital prominence with a genital flange on each side of the ventral face and a knob posteriorly and ventrally on the left lateral face. A small ridge extends across the ventral surface of the genital segment anterior to the prominence; it is slightly asymmetrical, being more pronounced on the
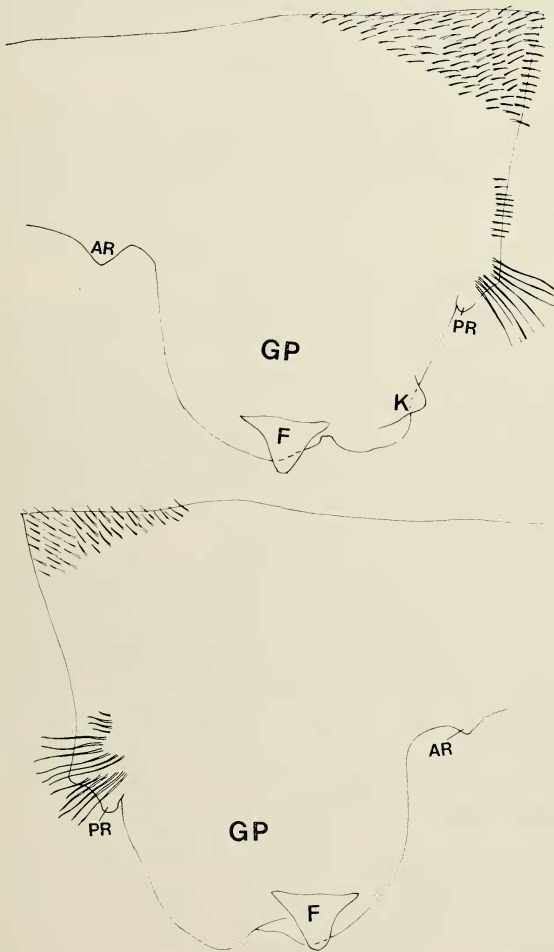
Fig. 1. Left (above) and right (below) lateral views of female genital segment. AR—anterior ridge; F—flange; GP—genital prominence; K—knob; PR—posterior ridge.

left side. Immediately posterior to the prominence is another large ridge with patches and rows of hairs on both sides. On the dorsal posterior region of the segment is another hirsute region.

I have recognized only 2 areas of spermatophore attachment. The first or 'correct' area is on or about the genital field, the ventral face of the genital prominence; this corresponds to grids A, D, F, and H of Hopkins & Machin (see their Fig. 1). The second or 'alternate' area is around, but

generally in front of the small ridge anterior to the genital prominence, which corresponds to grids B, E, and G of Hopkins & Machin.

Results

At DWDS *E. norvegica* is found between 400–1,000 m during the day; at night its distribution may extend to within 100 m of the surface. Seven hundred and ninety-five mature females (stage VI copepodids), 46 mature males, 73 immature females (stage IV & V copepodids) and 285 immature males were collected and examined. Spermatophores were found attached only to the genital segment of mature females.

Spermatophore placement.—Information concerning the number of adult females with and without spermatophores and the position of the latter is summarized in Table 1. Of the 795 females, 436 (55%) had at least one spermatophore. The number of spermatophores per female carrying at least one spermatophore is 1.3. The mean number of spermatophores per female is .72. This approaches the value of .80 reported by Hopkins and Machin during the July–August period, a peak period of spermatophore attachment for the Loch Etive population.

The 317 females with a single attached spermatophore represent 41% of the total population of females and 73% of those with at least one spermatophore. One hundred and forty-eight females (46%) have the spermatophore attached in the correct area while 169 (54%) have it in the alternate area. These data contrast rather sharply with the Loch Etive population. Hopkins and Machin report that for females carrying a single spermatophore, about 80% were placed in areas I would consider as the correct area (in their Fig. 7A add columns A, D, F, and H).

The 93 females with 2 spermatophores, representing 12% of all females and 21% of those with at least one spermatophore, are divided into 3 categories. Those with both spermatophores attached in the correct area constitute 10% of the total for double attachments. The 33 females with both spermatophores in the alternate area comprise 35% of the total double attachments. The majority, 55%, have one each in the correct and alternate positions. Thus, only 37% of all spermatophores on females with double attachments are found in the correct area. This figure is distinctly lower than that reported for Loch Etive. More important however is the low percentage of double placements in the correct position. Generally, if a female possesses two spermatophores, at least one will be found in the alternate area.

Finally, 26 females have 3 or 4 spermatophores. These make up only 6% of the females with at least one spermatophore and only 3% of the total females. Several combinations are possible in each category, but not all are represented (see Table 1). For example, no female has 3 or 4

Table 1. Numbers of females of *Euchaeta norvegica* with various combinations of spermatophores. S = number of spermatophores per female; T = total number of females; C = spermatophore in correct area; A = spermatophore in alternate area.

		Combinations of spermatophores													
S	T	1C	1A	2C	1A/ 1C	2A	3C	2C/ 1A	1C/ 2A	3A	4C	3C/ 1A	2C/ 2A	1C/ 3A	4A
0	359														
1	317	148	169												
2	93			9	51	33									
3	19						0	2	10	7					
4	7										0	0	1	4	2

spermatophores in the correct area. In fact, only 3 of the 26 have more than one spermatophore in the correct area.

Fertilization tubes.—Hopkins and Machin have described fertilization tubes in *E. norvegica* from Loch Etive. Such structures are also found on specimens from DWDS. In 2 instances fertilization tubes are found with spermatophores placed in the correct area, yet more anteriorly on the ventral face of the genital prominence. These tubes run to the genital opening. In the majority of cases, fertilization tubes are found in conjunction with spermatophores placed in the alternate area and connect these to the genital opening (Fig. 2). Of a total of 351 spermatophores placed in the alternate area (Table 2), 14% (59) have an associated fertilization tube. In terms of numbers of females, 279 have at least one spermatophore in the alternate position; about 18% (48 females) of these have at least one fertilization tube. On a single specimen, all 3 spermatophores in the alternate position possess fertilization tubes.

Of the 59 spermatophores with a fertilization tube, the spermatophore is empty in 51 cases. In the remainder, the outer contents are absent leaving only a center core of material in the spermatophore. Of the 237 correctly placed spermatophores, 7 are empty; included in this group are the 2 which had fertilization tubes.

The length of the fertilization tube is .22–.24 mm if, as usual, it follows a path over the anterior ventral ridge and down the anterior face of the genital prominence (as in Fig. 2). The tube is slightly longer in the few cases in which more than one spermatophore, each with a tube, has been placed in the alternate area. These other tubes then do not pass over the ridge but run across the left lateral side of the genital segment and down the left lateral face of the prominence.

Since only 14% of the spermatophores placed in the alternate area have fertilization tubes, it is possible, as stated by Hopkins and Machin, that
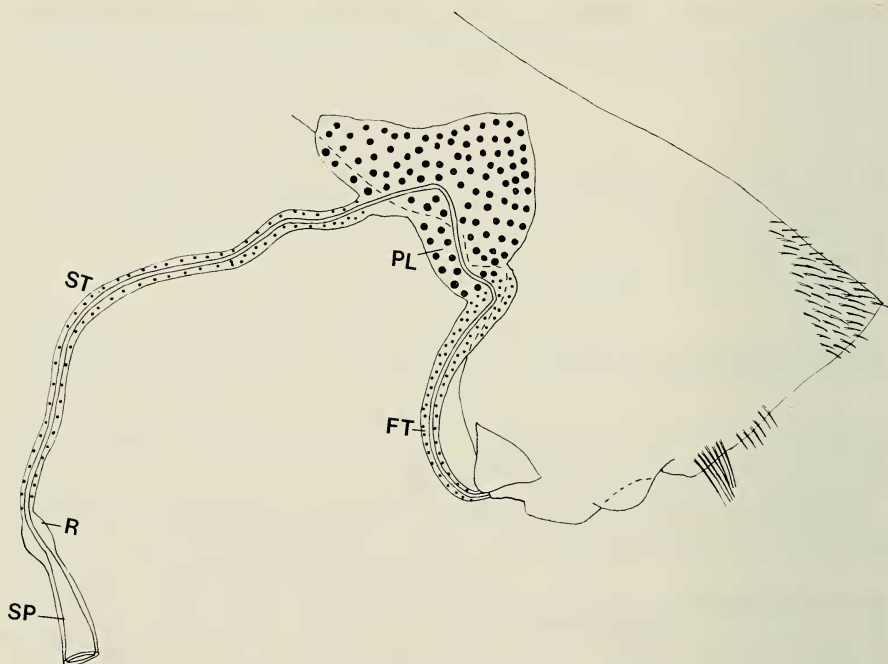
Fig. 2. Female genital segment, left lateral view, with stalk (ST) and plate (PL) complex and fertilization tubule (FT) of a spermatophore (SP) attached in alternate area. R—remnant of terminal spherical part of spermatophore.

few of the alternately placed spermatophores contribute to the fertilization of eggs. However, 88% (522) of all spermatophores (588) are full (Table 3). Seven in the correct area, including both with fertilization tubes are empty. All 51 in the alternate area which are empty have fertilization tubes associated. In the other 8 alternately placed spermatophores with fertilization tubes, the spermatophore is partly empty, lacking the matrix surrounding the central core in the spermatophore. Although alternately placed spermatophores with tubes represent only about 14% of that class, all are empty or partly empty, indicating that fertilization tubes are probably formed with all alternately placed spermatophores just before the contents of the spermatophore are emptied.

Males carrying spermatophores.—Forty-six males are found in the DWDS samples, about .06× the number of females. All are present in samples with females. All have spermatophores present in the bursa, or spermatophore sac, of the reproductive system (see Park, 1966, or Marshall & Orr, 1955, for a description of the anatomy of the male calanoid reproductive system).

Twenty-seven males, in addition to having one spermatophore in the

Table 2. Numbers of spermatophores in correct and alternate areas for single and multiple placements on females of *Euchaeta norvegica*.

	Correct	Alternate	Totals
Single placements	148	169	317
Double placements	69	117	186
Triple placements	14	43	57
Quadruple placements	6	22	28
Totals	237	351	588

reproductive system, hold a second spermatophore in the exopod of their left fifth leg (Fig. 3). It is possible to argue that these instances represent interrupted copulation during the time of sampling. However, if such is the case, it would seem improbable to find another spermatophore, virtually completely developed, in the bursa of these males. Instead, it appears that males of *E. norvegica*, like those of *Eurytemora affinis* (Katona, 1975) and *E. americana* (Williams, 1906), are able to secrete and carry one spermatophore with them before initiating direct physical contact with the female.

Changes in the spermatophore.—A comparison of the unattached spermatophore carried by the male fifth leg with those attached to the females shows interesting differences. A spermatophore removed from the fifth leg of the male is illustrated in Fig. 3. These range in size from .51–.55 mm (based on 10 examples). The spermatophore proper narrows to a short neck and then abruptly widens into a terminal spherical part, possibly the widened attachment disc plus a plug. The neck is enclosed by a short outer sleeve. The male grasps the spermatophore by the spherical part. It is held between the large claw of the serrated lamella and digitform process and is fronted by the third segment of the exopod of left fifth leg. By differential refraction of reflected light, the spermatophore proper can be seen to be divided into a central core and a surrounding matrix. The core is further differentiated at a point about ⅓ of its length from the neck. This may represent the dividing point between the proximal B-sper-

Table 3. Numbers of full, empty, and partly empty spermatophores on females of *Euchaeta norvegica*.

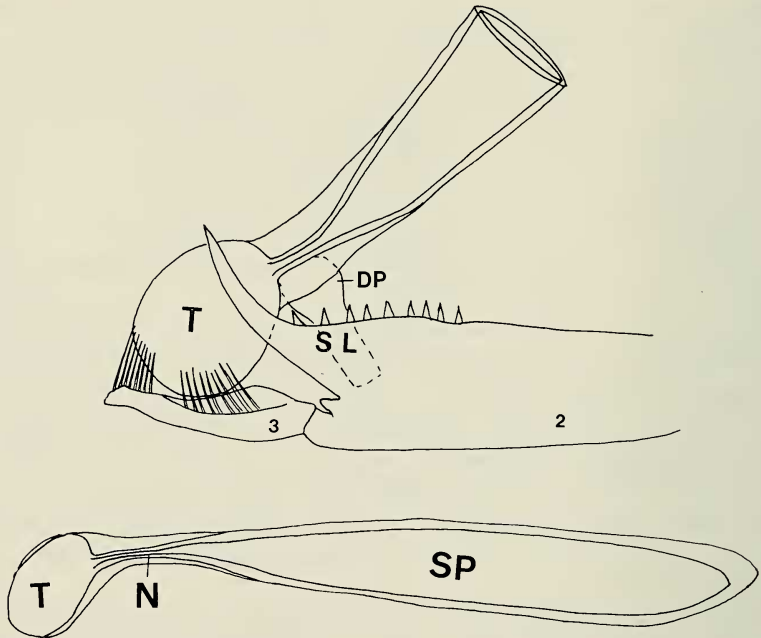|	Correct	Alternate	Totals
Full	230	292	522
Empty	7	51	58
Partly empty	0	8	8
Totals	237	351	588

Fig. 3. (Above) Terminal spherical part (T) of spermatophore held in second (2) and third (3) exopodal segments of male left fifth leg. (Below) Spermatophore removed from grasp of exopod of male left fifth leg. DP—digitiform process; N—neck; SL—serrated lamella; SP—spermatophore proper.

matozoa and the distal Q-spermatozoa (Heberer, 1932), the latter involved in forcing the former from the spermatophore. The core narrows to a conducting tube in the neck region.

Spermatophores attached to the female differ from the preceding by: 1) a reduction of the spherical part, the remnant of which can still be seen at a point below the neck (Fig. 2); 2) the development of a long stalk usually ending in a small disc at the point of attachment to the female genital segment. The disc, however, may be enlarged to an irregular plate when the spermatophore is attached in the alternate area. For spermatophores placed in the correct position, the length of the stalk varies from .38–.44 mm; those placed in the alternate area range from .50–.54 mm (both based on 10 examples).

Judging from the relative occurrences of these data, major changes can occur in the spermatophore after it has been secreted from the male reproductive system and picked up by the exopod of the left fifth leg. First, the stalk and plate complex forms, probably immediately before attachment for the plate and immediately before or after for the stalk. Ap-

parently after a relatively longer period of time, a fertilization tube forms if the spermatophore is placed away from the genital opening. Immediately after this, the spermatophore is emptied of its contents. The fertilization tube apparently forms after the male and female have separated and may develop as a phase change from sol to gel of the outer matrix of the spermatophore. Such a phase change has been observed in seminal fluids associated with copulation in *Centropages typicus* (Blades, 1977). The precise pathway of the fertilization tube may be determined by movement of the long hairs on the medial face of Th5 and the long seta on the basipodal segments of the fourth leg of the female.

Ratio of females to males.—The ratio of mature females to mature males is high (795:46) and does not reflect the number of spermatophores attached to females. One explanation is that by means of avoidance or loss through net meshes, males are collected less efficiently than females. The smaller size of the males (6.0–6.6 mm; females 7.4–8.6 mm) and their large, asymmetrical, hydrodynamically unstable fifth legs, however, would not seem to allow them to avoid a net more easily than the females. Loss through net mesh would be expected to differentially affect smaller specimens collected in a net. Although males of *E. norvegica* are smaller than females, they are still larger than most specimens collected in this study (unpublished data). Differential loss through the net mesh is probably not significant enough to explain differences between numbers of females and males.

A second explanation is suggested by the ratio of immature females to immature males (73:285). Here it would seem that there are enough future mature males to provide spermatophores for all future mature females. It is possible that males and females spend the same amount of time at each stage of development through copepodid V. As mature animals (copepodid VI), the males spend much less time than females, i.e. they have a much lower turnover time. This conclusion is supported by the fact that males do not feed as mature animals. The mandibular gnathobase and first lobe of the maxillule, well developed in copepodid V, are lost in copepodid VI. After mating once, or possibly several times, the males probably die. In the females the well developed mouthparts are retained in copepodid VI and feeding undoubtedly continues in this stage to provide energy for later egg development.

Discussion

The preceding observations of spermatophore placement in *E. norvegica* pose 4 important questions. Why are some spermatophores placed in the alternate area? What is the function of such alternately placed spermatophores? Why is more than one spermatophore attached? How can the

evolution of such phenomena be explained within the context of a speciation process which places a high premium on precision and accuracy in all aspects of mating behavior (Fleminger, 1975)?

Concerning the function of alternately placed spermatophores, Hopkins and Machin state "the majority of imperfectly positioned spermatophores have no connexion between the attachment disc and the females (sic) seminal receptacles and cannot be thought of as viable for fertilization." Later they note that only those with fertilization tubes are fully viable. In samples from DWDS, as noted, the percentage of alternately placed spermatophores without tubes is very high, 86%. If these are not potentially viable, they would seem to represent quite a waste of energy. However, in all cases where fertilization tubes connect alternately placed spermatophores to the genital opening, the associated spermatophores are empty or partly empty. It is possible that all alternately placed spermatophores are capable of forming fertilization tubes and that tube formation occurs some time after spermatophore attachment and immediately before the spermatophore is emptied and subsequently removed by the female. This would explain why all specimens with fertilization tubes had empty or partly empty spermatophores. Because of delayed formation, fertilization tubes need not be associated with all such spermatophores at any one time.

Hopkins & Machin suggested tactile recognition of the female *E. norvegica* by the male using the ciliated area of the exopod of the left fifth leg. This idea is intuitively very appealing. Yet ciliated areas of the exopod of the male fifth leg are found in many calanoid families; besides Euchaetidae, for example the Pseudocalanidae, Aetideidae, Scolecithricidae, and Phaennidae. The primary function of this ciliated area seems to be to grasp the spermatophore while it is being transferred to the female. It may also be involved in a tactile interrogation of the female genital segment but this function would have to be carried out in *E. norvegica* while the male is holding the spermatophore since there is evidence that the male secretes and grasps the spermatophore before making direct contact with the female. Nevertheless, assuming that mating behavior involves the careful choice of position of attachment by the male and that most alternately placed spermatophores are viable via fertilization tubes, the question still arises, why place a second or third spermatophore in an alternate area? Why not, as has been observed in *Eurytemora affinis* (Katona, 1975) and *Acartia tonsa* (Hammer, in press), place those spermatophores in the correct area, that is just as close to the genital opening as the first?

I have assumed a different attachment behavior for *Euchaeta norvegica.* After mounting and positioning himself on the female, the male attempts to attach the spermatophore in the correct area with a quick stroke of the

left fifth leg. Potential points of attachment then are restricted by the mechanical considerations of the length of the exopod of the left fifth leg and the flexion allowed by each segment of the exopod once the initial position with respect to the female has been determined. Then a spermatophore in the alternate area simply represents the result of a mechanical error, possibly caused by an attempt to attach it before the final correct position on the female was achieved. Hopkins & Machin suggested that until the female genital opening is sealed either by a spermatophore or a fertilization tube, the female may still be mated. Thus the position of the initial spermatophore very definitely affects the position of additional spermatophores since a female with an alternately placed spermatophore in which a fertilization tube has yet to form, can be mated again. Their suggestion would also explain the very low percentage, 10%, of 2 or more spermatophores in the correct area in multiple placements, since a correctly placed spermatophore would close the genital opening.

No matter which attachment behavior is choosen, the final question remains: how could a system of multiple spermatophores and misplaced attachments, either by choice or chance, have evolved within a speciation process which places a premium on precision in the morphology of structures associated with mating and accuracy in mating behavior? The systematic importance of the morphology of segments and appendages which function in mating behavior of copepods has been demonstrated for *Centropages* by Lee (1972). Steuer (1924) suggested that the morphological diversity in the female genital segment of *Acartia* acts as an obstacle to hybridization in closely related species. A complete study of these structures associated with prezygotic mating barriers in the genus *Labidocera* and an elegant statement of the process has been made by Fleminger (1975).

Mating behavior can be envisioned as a linear sequence of exact, time-ordered events. Each event has a very high probability, approaching unity, of occurring between sexes of the same species and a reduced probability between sexes of differing species. These probabilities are insured by precise morphological, physiological, and behavioral adaptions associated with each event. During incipient speciation, if prezygotic mating barriers have resulted in biological isolation then the sum total of all probabilities should approach zero for pairings with sexes of the different daughter populations. As the evolution of the daughter species continues, it is possible that biological isolation may be preempted by a particular subset of the initial set of events. Then the accuracy of events before or after that subset, but especially farther along the time sequence, might be relaxed. This, then, may explain how imprecision in spermatophore placement might have initially developed in *Euchaeta norvegica*.

Summary

The total number and relative numbers of single, double, triple, and quadruple placements of spermatophores on females of *Euchaeta norvegica* from samples collected in the Deepwater Dumpsite 106 agree with the data reported for Loch Etive, Scotland by Hopkins & Machin (1977). However, the Dumpsite population differs in having a higher number of spermatophores placed away from the ventral face of the genital prominence in an alternate area of attachment anterior to the genital prominence. The proportion of adult males was low; however, there were many more stage IV & V males than females. Some adult males were found clasping a spermatophore in the exopod of the left fifth leg. This spermatophore differs from those attached to the female by the addition of a long stalk, and attachment plate. A fertilization tube may be found associated with any spermatophore placed away from the female genital opening, but usually with those in the alternate area. Spermatophores attached in the alternate area with fertilization tubes were never completely full. The formation of the tube is believed to occur sometime after initial attachment but immediately before the spermatophore is emptied. Within the context of a speciation process which emphasizes morphological precision and behavioral accuracy, the variability in spermatophore placement is explained by hypothesizing the preemption of biological isolation by a subset of events in the initial time-ordered sequence of behavioral events involved in the isolation and a subsequent relaxation of events outside of the subset.

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